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Review Article

## Smoking and periodontal microorganisms

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### SUMMARY

Resolution of dysbiosis following treatment for periodontal disease and tobacco dependence has been reported in longitudinal intervention studies. In the present report, we evaluated the biological findings regarding the effect of smoking on the periodontal microbiome. A standardized electronic search was conducted using MEDLINE; overall, 1099 papers were extracted. Studies that addressed the relationship between tobacco and periodontal pathogens were included. Finally, 42 papers were deemed appropriate for the present review. Functional changes in periodontal pathogens exposed to nicotine and cigarette smoke extract support the clinical findings regarding dysbiosis of the subgingival microbiome. Dysbiosis of the periodontal microbiome was presented in smokers regardless of their periodontal condition (healthy, gingivitis, or periodontitis) and remained significant only in smokers even after the resolution of experimentally-induced gingivitis and following reduction of clinical signs of periodontitis with non-surgical periodontal treatment and over 3 months post-therapy. Based on these findings, smoking cessation in periodontitis patients is beneficial for promoting a health-compatible subgingival microbial community. To maximize the benefits of these interventions in dental settings, further studies on periodontal microbiome are needed to elucidate the impact of tobacco intervention on preventing recurrence of periodontal destruction in the susceptible subjects.

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### 1. Introduction

Smoking and exposure to secondhand smoke causes various human diseases. The primary reasons for global tobacco control are based on evidence of causality between exposure to tobacco

smoke and disease. Categories for judgment of causal inference were applied to oral diseases in national reports such as the 2014 Surgeon General's Report in the US [1] and a report on the health consequences of smoking of the Review Meeting of Ministry of Health, Labor and Welfare in Japan, published in 2017 [2] (Table 1). Cancers of the oral cavity and pharynx and periodontitis are among the diseases that have been causally linked to smoking. Orofacial clefts are congenital defects causally linked to maternal smoking. This disease was included only in the US report. Other diseases of

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**Table 1**

Level of evidence to infer causality between active smoking and oral diseases that was included in the national reports of the US in 2014 and Japan in 2017.

Level of evidence	Oral diseases
Sufficient	Cancer of oropharynx Periodontitis Orofacial clefts*
Suggestive	Dental caries Failure of dental implants Tooth loss**

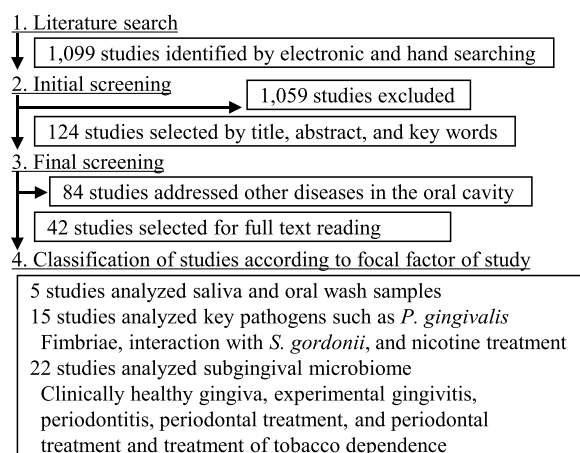
Reported only in the US\* and Japan\*\*.

the oral cavity are categorized in the lower level of causal inference; evidence is suggestive but not sufficient to infer causal relationships between active cigarette smoking and dental caries and failure of dental implants, and between exposure to tobacco smoke and dental caries in children. An association between active cigarette smoking and tooth loss was categorized in this level of causal inference in the Japanese report, but not mentioned at all in the US report.

Beyond the epidemiological evidence, biological plausibility is one of the major elements of causal inference [3]; the observed relationship between smoking and disease should be coherent or plausible in terms of known scientific principles, biological mechanisms, and observed patterns of disease. Many studies have addressed potential mechanisms linking smoking and periodontitis, including molecular and genetic factors in addition to impairment of wound healing [4]. Given the obvious association of smoking with destructive periodontal inflammation, a more detailed assessment of the effects on periodontal pathogens is warranted. In a review article investigating the potential mechanisms linking smoking and oral and upper respiratory tract bacterial pathogens, findings regarding the impact of exposure to smoking and smoking cessation on the flora of otitis media and the nasopharynx, respectively, were strengthened in relation to the association between reduction in the numbers of species of the normal oral mucosa flora and a greater frequency of respiratory infections [5]. New technology for microbiome detection has been applied to oral samples [6]. To the best of our knowledge, there are few review articles addressing the principal mechanisms that explain the relationships between smoking and periodontal microorganisms. In this review, we focus on the biological findings of the relationship between exposure to tobacco smoke and periodontal microorganisms.

## 2. Review process

We conducted a literature search to examine the relationship between smoking and oral microorganisms. Of the identified literature, studies on periodontal disease-related bacteria were extracted. The remaining studies were preserved for a separate review. The literature search for oral microorganisms was divided into two topics: periodontal disease and other diseases of the oral cavity. The present literature review addresses oral microorganisms relevant to periodontal disease. An electronic search of English language publications was conducted using MEDLINE (December 2007 to November 2017) to identify pertinent literature. The search strategy applied was as follows: (nicotine OR smoking OR smoking cessation OR tobacco) AND (dental caries OR dental implants OR gingivitis OR oral health OR oral hygiene OR peri-implantitis OR periodontal disease OR saliva) AND (bacteria OR dental plaque OR ecosystem OR human papilloma virus OR HPV OR microbiome OR oral microbiology). Search results were selected based on their title and abstract and stored using literature management software (Endnote X8, Clarivate Analytics, Philadelphia, PA) for initial screening. Two calibrated reviewers screened the results inde-



**Fig. 1.** Number of studies found by our search and selection. The literature search for oral microorganisms consists of two parts: periodontal disease and other diseases of the oral cavity. The present review addresses oral microorganisms relevant to periodontal disease. The remaining studies were preserved for a separate review.

pendently. Disagreements between reviewers were resolved by discussion until a consensus was reached. Final screening was conducted by full-text reading, and articles were classified according to the relevant oral diseases. The reference lists of articles that were read in full were also considered. Epidemiological studies not relating to oral microorganisms were excluded. In total, 1099 articles were identified and classified according to oral disease-associated microorganisms. These studies addressed periodontal pathogens as well as other microorganisms including human papilloma virus, *Candida* species, *Streptococcus mutans*, and peri-implant microorganisms. Among these papers, 42 were deemed appropriate for this review of the relationship between smoking and periodontal microorganisms: 5 studies analyzed saliva and oral wash samples, 15 studies analyzed key periodontal pathogens such as *Porphyromonas gingivalis*, and the remaining 22 studies analyzed the subgingival microbiome. Special attention was given to microorganism detection methods such as colony counting, quantitative polymerase chain reaction (qPCR), checkerboard DNA hybridization, microarray, and 16S rRNA sequencing (Fig. 1).

## 3. Microorganisms in saliva and oral wash samples from smokers

Microorganisms in saliva include bacteria from the surfaces of different oral tissues. Microarray analysis of stimulated saliva from 292 individuals with low levels of dental caries and periodontal disease showed a higher abundance of two bacterial taxa, *S. sobrinus* and *Eubacterium brachy*, in smokers as compared with nonsmokers [7]. Next-generation high-throughput sequencing of saliva samples from 2343 cohorts of a Japanese provincial town showed that higher phylogenetic diversity was associated with poorer oral health and current smoking. Current smoking was associated with the relative abundance profile of 13 bacterial genera including *Prevotella* and *Veillonella* among the core bacterial species as characterized by a higher ratio of the operational taxonomic units [8]. 16S rRNA sequencing of oral wash samples from 1205 US adults showed differences in the overall microbiome composition between current and non-current smokers, and functional analysis from inferred metagenomes revealed that bacterial genera related to carbohydrate, energy, and xenobiotic metabolism were depleted by smoking [9]. These findings suggest the potential role of smoking in the shift in functions of the oral microbiome in smoking-related oral diseases.

Bacterial sequencing of oral wash and bronchoscopic alveolar lavage samples from 19 smokers and 45 nonsmokers indicated dif-

ferences in oral microbial communities in smokers, but the lung microbiome was not significantly different [10]. 16S rRNA sequencing of supra and subgingival plaque, saliva, soft oral tissue, and nasal swab samples from 23 current smokers and 20 nonsmokers showed lower alpha diversity in smokers than in nonsmokers in the buccal mucosa, but samples from other sites showed no significant differences in microbial diversity and composition [11]. These findings implicate alteration of the microbiome in the oral cavity and respiratory tract due to smoking. The periodontal microbiome is the potential origin of the oral microbiome that is altered by smoking, which was presented earlier in intervention studies of treatments of periodontal disease and tobacco dependence [12,13].

#### 4. Analysis of key periodontal pathogens

##### 4.1. Effects of exposure to cigarette smoke extract (CSE) on *P. gingivalis* fimbriae

Effects of exposure to cigarette smoke extract (CSE) on *P. gingivalis* fimbriae

*P. gingivalis* adapts to CSE, an environmental stress, by altering the expression of its major fimbrial antigen in addition to its capsule [14]. CSE upregulates the *P. gingivalis* major fimbrial antigen while suppressing polysaccharide production in the capsule that promotes *P. gingivalis* colonization and infection [15]. *S. gordonii*, an early colonizer in oral cavities, interacts with *P. gingivalis*. CSE exposure promoted the formation of a *P. gingivalis*-*S. gordonii* biofilm in a dose-dependent manner, with a stronger *P. gingivalis* major fimbriae binding capacity than the control, but did not induce *P. gingivalis* auto-aggregation [16]. Alterations to *P. gingivalis* fimbriae caused by exposure to CSE were completely reversed when CSE-exposed *P. gingivalis* was subcultured in fresh medium without CSE [14]. These effects on the *P. gingivalis* phenotype could explain the alterations that promote colonization and infection by key periodontal pathogens, and the reversibility of the fimbrial alteration observed *in vitro* suggests that there are benefits to smoking cessation.

##### 4.2. Effects of nicotine treatment on *P. gingivalis*

Treatment with nicotine at a range of concentrations in saliva of smokers, from 0 to over 2 mg/ml stimulated *S. gordonii* planktonic cell growth, increased biofilm formation and bacterial cell mass, and upregulated *S. gordonii* binding-related genes in planktonic cells [17]. Treatment of *S. gordonii* with nicotine may contribute to *P. gingivalis*-*S. gordonii* biofilm formation. However, the role of nicotine on *P. gingivalis* growth is not straightforward. Growth of *P. gingivalis* was inhibited by nicotine treatment in a dose-dependent manner by a single exposure method, but its growth rate increased with each subsequent exposure to nicotine in a multiple-treatment model [18]. Exposure to 0.1 mg/ml of cotinine, a metabolite of nicotine, increased *P. gingivalis* association and invasion of epithelial cells, but this was not affected by exposure to nicotine [19]. Inoculation of primary epithelial cell monolayers with nicotine and cotinine at a concentration of 1 mg/ml increased colonization of *Aggregatibacter actinomycetemcomitans* in a time-dependent manner, but decreased colonization of *P. gingivalis* [20]. Nicotine and its metabolite cotinine may promote the formation of pathogenic biofilms by enhancing the *P. gingivalis*-*S. gordonii* interaction, but further research is needed to clarify and explain the role of nicotine on *P. gingivalis* growth.

##### 4.3. Effects on microbial functions for pathogen-host interaction

*P. gingivalis* adapted to the environmental stress of CSE exposure by altering gene expression and outer membrane proteins

[14]. CSE changed the *P. gingivalis* phenotype so as to suppress production of capsular polysaccharides at the ultrastructural level and to neutralize the pro-inflammatory response to subsequent TLR2 stimulation [15]. *P. gingivalis* biofilms grown in the presence of CSE exhibited a lower pro-inflammatory capacity (involving TNF- $\alpha$ , IL-6, and IL-12) than control biofilms [14,16]. Most effects were reversed when CSE-exposed bacteria were subcultured in fresh medium without CSE [14]. Treatment of *Campylobacter sputigena* with smokeless tobacco extracts caused metabolic alterations due to oxidative stress [21]. A combined treatment of human gingival cells with CSE and *P. gingivalis* was then examined. Treatment of fibroblasts with CSE and *P. gingivalis* supernatant destroyed the balance between matrix metalloproteinases and their tissue inhibitors [22]. The effects of combined treatment of dendritic cells (DCs) with nicotine and *P. gingivalis* lipopolysaccharide on the immunopathogenesis of periodontal diseases suggested modulation of the immunoregulatory functions of *P. gingivalis* LPS-stimulated DCs [23]. Combined treatment of gingival epithelial cells with low concentrations of CSE and *P. gingivalis* inhibited wound closure, and *P. gingivalis* invasion increased near the wound area [24]. Clinically, smoking was associated with decreased total IgG response against *P. gingivalis* strains but did not influence IgG produced against specific cell-surface proteins, indicating that smoking alters the humoral response against *P. gingivalis* and may increase *P. gingivalis* infectivity [25]. Smoking tends to reduce the effectiveness of the immune response regarding *P. gingivalis* antibodies in the blood serum of patients with periodontitis [26]. The pronounced cross-talk between clinical variables and antibody profiles in smokers with periodontitis may be mediated by modulation of the *P. gingivalis* antibody response profile [27]. Further indirect evidence includes reductions in 3-OH fatty acids in whole saliva samples, which is consistent with an oral microflora of reduced inflammatory potential, from smokers compared with those from nonsmokers with chronic periodontitis [28]. It is well recognized that smoking reduces the host response to major mucosal pathogens such as *P. gingivalis*. Such alterations of microbial functions due to tobacco exposure may contribute to the reduced host response to periodontal pathogens.

#### 4.4. Summary

To elucidate the biological pathways of effects of exposure to smoking on periodontal disease, periodontal pathogens were exposed to CSE, smokeless tobacco extract, or nicotine *in vitro*. CSE alters the expression of major fimbrial antigen and promotes colonization and infection of a key periodontal pathogen, *P. gingivalis*. Exposure to nicotine could modulate immunological function via *P. gingivalis* and promote biofilm formation via attachment to the commensal oral bacteria *S. gordonii*. Potential pathways of tobacco smoke exposure to periodontal destruction may be partially explained by the modulation of pro-inflammatory and antibody responses and by the combined treatment of human cells with tobacco constituents and periodontal pathogens. Overall, exposure to smoking could promote pathogenesis of a periodontal biofilm via alterations of *P. gingivalis* properties such as biofilm formation and functions related to pathogen-host interaction.

### 5. Subgingival microbiome in smokers

#### 5.1. Clinically healthy gingiva

More than 1.6 million sequences from subgingival samples from 200 systemically and periodontally healthy smokers and nonsmokers were analyzed by 16S rRNA sequencing. Microbial profiles were different between smokers and nonsmokers at all taxonomic lev-

els, and clustering of the microbial communities was distinct in smokers, representing a highly diverse, pathogen-rich, commensal-poor, anaerobic microbiome, which is associated with the microbial community of periodontitis patients [29]. Marginal and subgingival biofilm samples following 7 days of undisturbed plaque formation in 15 smokers and 15 nonsmokers were analyzed by 16S rRNA sequencing. Smokers showed early colonization of periodontal and respiratory pathogens and a highly diverse, relatively unstable initial colonization compared with nonsmokers, and proinflammatory cytokines in the gingival crevicular fluid samples was increased in smokers compared with nonsmokers. Increased levels of proinflammatory cytokine were positively associated with commensal microbiota in smokers, but this association was not observed in nonsmokers [30]. Authors stated that smoking creates “an at-risk-for-harm microbial community” in the oral biofilm [29,30].

### 5.2. Experimental gingivitis

Subgingival plaque samples from 15 young smokers were analyzed by 16S rRNA sequencing during the onset and resolution of experimental gingivitis. Each treatment was administered for 21 days. The abundance of early colonizers such as *Streptococcus*, *Veillonella*, and *Pseudomonas* decreased, and the levels of periodontal pathogens such as *Treponema*, *Selenomonas*, *Parvimonas*, *Dialister*, and *Campylobacter* increased, followed by clinical signs of gingivitis. The shift in the microbiome was accompanied by a temporary decrease in certain mediators of anti-inflammatory, chemokine, and T-helper 2 responses on day 7, but most inflammatory responses had increased even after the resolution of clinical gingivitis and restoration of bacterial communities to healthy levels. These results could be explained by the characteristics of early acquisition of pathogenic microorganisms in the marginal and subgingival biofilms of smokers, which elicit a sustained host response that persists even after removal of the bacterial challenge [31]. Experimental gingivitis was induced for 21 days in healthy dental students, 10 smokers and 14 nonsmokers. Both smoking groups showed development of gingival inflammation excepting less bleeding in the gingiva of smokers and restoration to baseline levels after resumption of oral hygiene. Based on checkerboard DNA-DNA hybridization, smokers presented higher total bacterial counts and higher proportions of red and orange complex bacteria and lower proportions of *Actinomyces* species, and of purple and yellow complex bacteria in the supragingival plaque samples than nonsmokers [32]. After a 7–10 day period of resolution of pre-existing gingivitis in 25 smokers and 25 nonsmokers, experimental gingivitis was induced for 21 days followed by a secondary 7–10 day resolution period. Changes in the bacterial composition in marginal and subgingival plaque samples and pro-inflammatory responses were evaluated by 16S rRNA sequencing and measurement of 27 immune mediators in the gingival crevicular fluid samples, respectively. Compared with nonsmokers, smokers demonstrated earlier colonization of periodontal and respiratory pathogens with corresponding immune responses followed by a greater abundance of pathogenic species, poorer connections between marginal and subgingival ecosystems, and greater pro-inflammatory responses persisting during the second resolution phase. The authors stated that the reduced ability of the subgingival microbiome to “reset” itself in smokers compared with in nonsmokers following episodes of the disease may lead to lowered resilience of the gingival ecosystem and a decreased ability to resist future disease in smokers [33]. Studies of experimental gingivitis demonstrated relatively early acquisition of periodontal and respiratory pathogens in the marginal and subgingival biofilms of smokers and impaired ecosystem recovery accompanying a prolonged and sustained immune response, even following resolution of the clinical aspects of inflammation. These findings provide evidence of lowered ecosystem

resilience in smokers. The observation of a temporal decrease in immune system mediators observed in smokers during the early phase of experimental gingivitis may require further research to clarify the potential relationship between decreased immunoglobulin production and *P. gingivalis* exposure to CSE.

### 5.3. Periodontitis

Based on the results of qPCR, 40 smokers showed significantly higher amounts of *P. gingivalis*, *A. actinomycetemcomitans*, and *T. forsythia* than 40 nonsmokers in chronic periodontitis pockets with equal probing depths. Smoking was associated with the presence of *A. actinomycetemcomitans* [34]. Other studies using the qPCR detected small or no significant differences in the composition of microorganisms in the subgingival plaque samples [35–37]. Composition of the subgingival microbiome as evaluated by 16S rRNA sequencing was different between 15 smokers and 15 nonsmokers with periodontitis, but the microbial techniques used, including culturing and qPCR, did not specifically identify the difference. The microbial profile of subgingival plaque samples from periodontitis smokers was distinct from that of nonsmokers: the genera *Fusobacterium*, *Prevotella*, and *Selenomonas* were more abundant in smokers, while *Peptococcus* and *Capnocytophaga* were more abundant in nonsmokers [38]. In subgingival plaque samples from deep sites of 15 smokers and 15 age- and sex-matched nonsmokers, 16S rRNA sequencing showed a greater abundance of *Parvimonas*, *Fusobacterium*, *Campylobacter*, *Bacteroides*, and *Treponema* and lower levels of *Veillonella*, *Neisseria*, and *Streptococcus* in smokers than nonsmokers. Among uncultivated microorganisms, the levels of several species such as *Peptostreptococci*, *Parvimonas micra*, *Campylobacter gracilis*, *Treponema socranskii*, *Dialister pneumosintes*, and *Tannerella forsythia* were elevated, while *Veillonella* sp. oral clone B2, *Neisseria* sp. oral clone 2.24, *S. sanguinis*, and *Capnocytophaga* sp. clone AH015 were less abundant in smokers than in nonsmokers [39]. Differences in the subgingival bacterial community between smokers and nonsmokers with periodontitis were reported in Korean patients [40].

Smoking has been reported to be among the factors which may influence the subgingival microbiome. Subgingival plaque samples from 12 patients with chronic periodontitis showed a high relative abundance of *Parvimonans*, *Desulfubulbus*, *Paludibacter*, *Haemophilus*, and *Sphaerochaeta* compared with those from 8 healthy controls. Among the periodontitis patients, a major microbial community alteration was evident in 6 smokers when compared with 6 nonsmokers [41]. Subgingival plaque samples taken from deep sites were different in abundance of 51 genera and 200 species compared with samples taken from shallow pockets in 88 patients with chronic periodontitis. Differences in the microbiome between deep and shallow sites were influenced independently by smoking among patient-level factors [42], indicating that smoking independently influences the difference in microbiome by pocket depth in periodontitis patients. The synergistic effect of smoking and hyperglycemia on disease-associated subgingival microbiomes was greater than sum of each individual effect [43].

16S rRNA sequencing analysis of subgingival plaque samples demonstrated differences in the composition of the subgingival microbiome between smokers and nonsmokers [5,39–41,44]. These findings were further confirmed by baseline data from intervention studies [12,13,43]. The findings suggest an association between smoking and subgingival dysbiosis. Smoking may enrich the pathogenic microbial community in subgingival plaque and reduce the abundance of healthy commensals, although intervention studies on experimental periodontitis and initiation of smoking in humans are limited. Aside from this limitation due to

ethical consideration, intervention studies on the effects of periodontal treatment and smoking cessation are feasible.

#### 5.4. Periodontal treatment

Checkerboard DNA-DNA hybridization was used to detect changes in periodontal pathogens following periodontal treatment. Clinical parameters in 40 smokers and 40 nonsmokers with chronic periodontitis improved during a follow-up period of 3 months after non-surgical and surgical periodontal therapy. Smoking was associated with clinical signs of deep pockets and less bleeding upon probing. However, a significant reduction in red and orange complex species was observed in nonsmokers only [45]. The effects of nonsurgical periodontal treatment, including scaling and root planing, and nonsurgical periodontal treatment, including adjunctive antimicrobial photodynamic therapy, were compared between 20 smokers with chronic periodontitis by using a split-mouth experimental design. Neither periodontal treatment regimen affected the levels of 40 bacterial species in the subgingival plaque samples during the follow-up period of 12 weeks post-therapy, indicating that smoking may impair periodontal healing after nonsurgical treatment [46]. The weaker effect of periodontal treatment on periodontal pathogens relative to clinical measures in smokers was also confirmed by 16S rRNA sequencing. Before supragingival periodontal therapy for severe chronic periodontitis, 10 smokers showed a higher proportion of several pathogens and a lower number of cultivated phylotypes than 10 nonsmokers. More modest changes in the periodontitis-associated phylotypes were observed 21 days after therapy in smokers compared with nonsmokers. However, smokers and nonsmokers alike showed significant changes in clinical parameters with no significant difference between groups. Supragingival therapy only slightly affected the subgingival biofilm biodiversity in smokers as compared with nonsmokers [43]. Following nonsurgical treatment with scaling and root planing over a period of 21 days, 15 smokers and 15 nonsmokers showed clinical improvement, a decrease in three major periodontal pathogens, and an increase in the proportion of host-compatible species in the subgingival plaque samples. During the 180-day post-therapy follow-up period, however, subgingival bacterial recolonization of the pathogenic species was observed only in smokers. Smokers appear more susceptible to re-establishment of a pathogenic subgingival biofilm than nonsmokers [47]. Compared with the improvement of clinical signs of periodontitis in both smokers and nonsmokers after periodontal treatment, the response of periodontal microbiome to periodontal therapy remained poor in smokers. This finding suggests the poor response in periodontal pathogens of smokers is associated with impairment of periodontal healing observed during treatment with periodontitis.

#### 5.5. Periodontal treatment and treatment of tobacco dependence

Periodontal nonsurgical management with root planing and smoking cessation counseling were prescribed to 22 patients with chronic periodontitis. Among them, 11 patients quit smoking and the other continued to smoke. At the examination period of baseline, 3, 6 and 12 months post-treatment, subgingival plaque samples were analyzed by the 16S rRNA sequencing for microbial community. Quitters demonstrated divergent profiles of microbial community following smoking cessation. Microbial profiles at 6 and 12 months were different between groups. Microbial profiles of smokers at post-treatment periods remained similar to baseline [12]. In the subgingival plaque samples of quitters, prevalence of *P. endodontalis* and *Dialister pneumosintes*, and levels of *Parvimonas micra*, *Filifactor alocis*, and *Treponema denticola* were decreased, and level of *Veillonella parvula* was increased. Quitters exhibited a greater number of health-associated species and lower preva-

lence and abundance of putative periodontal pathogens compared with that at baseline [13]. The apparent difference in the shift of microbial profile in the subgingival microbial community between quitters and smokers following nonsurgical periodontal therapy and smoking cessation indicates benefit of treatment of tobacco dependence that may contribute to prevention of development of periodontal disease by altering subgingival ecosystem toward a health-compatible profile of microbiome [12,13]. Further studies are needed to elucidate the role of the interventions with surgical periodontal therapy and treatment of tobacco dependence for the prevention of development of periodontal diseases.

#### 5.6. Summary

Since next-generation high-throughput 16S rRNA sequencing began to be used to analyze subgingival plaque samples to explore the compositional and functional changes in the microbiome of smokers [6], evidence has steadily accumulated regarding the role of non-surgical treatment and tobacco interventions in dental settings. Changes in the microbial community in subgingival plaque samples with respect to periodontal disease-associated and health compatible species have been demonstrated. These findings extend and confirm previous knowledge obtained by the analyses by traditional molecular approaches such as qPCR and checkerboard DNA-DNA hybridization. Studies of the effects of treatments for periodontal diseases and tobacco dependence on periodontal pathogens with randomized controlled trials are scarce, potentially because of low rates of study completion due to low rates of successfully quitting smoking. However, importantly, any chance of smoking cessation must not be missed because tobacco cessation intervention by health care professionals is effective at increasing the success of attempts to quit [48,49]. Tobacco dependence treatment should be offered to smokers with periodontal disease to decrease the risk of development of periodontal disease. Further intervention studies for different populations with randomized controlled designs are needed to confirm the benefit of smoking cessation in preventing development of periodontal destruction by alteration of the subgingival microbial community.

### 6. Effects of tobacco use on oral microorganisms not included in the periodontitis-associated microbiome

During the construction phase of a searching strategy for relevant literature regarding the effect of tobacco use on oral microorganisms, we noticed that the focal microorganism not included in the periodontitis-associated microbiome were human papilloma virus, *Streptococcus mutans*, *Candida* species, and peri-implant microorganisms. The potential effect of tobacco use on HPV infection was intensively studied, based on findings of observational epidemiological studies. Effects of nicotine exposure on biofilm formation were investigated for *S. mutans*, *S. sanguinis*, and *Candida* species. Oral apparatus such as oral implants, removal denture, and various materials used for orthodontic treatment were focal factors for the effects of exposure to tobacco constituents on the biofilm formation. The effect of tobacco use on these microorganisms must be addressed comprehensively in addition to periodontal pathogens in line with tobacco interventions by oral health professionals for oral and overall health of dental patients not only at the specific disease level but also at a personal level.

### 7. Conclusions and implication for future study

The biological findings of the relationships regarding exposure to tobacco smoke, organized by sample types and study conditions,

**Table 2**  
Biological findings according to types of sample in the study and study conditions.

Samples	Study conditions	Biological findings
Saliva and oral wash	Smokers and nonsmokers	Alteration of oral microbiome due to smoking, shift of oral microbiome functions in smoking-related oral diseases, and periodontal microbiome as the potential origin of oral microbiome altered by smoking.
Key periodontal pathogens	Effects of exposure to cigarette smoke extract on <i>P. gingivalis</i> fimbriae	Alterations of <i>P. gingivalis</i> that promote colonization and infection and benefit of smoking cessation because of reversibility of the fimbrial alteration.
	Effects of nicotine treatment on <i>P. gingivalis</i>	Promotion of pathogenic biofilm formation by enhancing the <i>P. gingivalis</i> - <i>S. gordonii</i> interaction.
Subgingival plaque	Effects of exposure on inflammatory response	Reduction of host response to major mucosal pathogens such as <i>P. gingivalis</i> by altering pathogen-host interaction.
	Clinically healthy gingiva	Dysbiotic oral bacterial communities in the oral biofilms of smokers with clinically healthy periodontal tissue.
	Experimentally-induced gingivitis	Lowered ecosystem resilience in smokers.
	Periodontitis	Association between smoking and subgingival dysbiosis.
	Periodontal treatment	Dysbiosis as the cause of impairment of periodontal healing.
	Periodontal treatment and treatment of tobacco dependence	Benefit of treatment of tobacco dependence for prevention of periodontal disease progression by altering subgingival ecosystem toward a health-compatible profile of microbiome.

are summarized in Table 2. Analyses of microbiome in saliva and oral wash samples implicate that the periodontal microbiome is the potential origin of alterations in the oral microbiome resulting from smoking. Nicotine treatment and exposure to cigarette smoke extract alter the function of key periodontal pathogens such as *P. gingivalis* and promote biofilm formation, colonization, and infection. New 16S rRNA sequencing technology has shed new light on the subgingival microbiome of smokers and quitters to oral health professionals. Dysbiosis of the periodontal microbiome was observed in smokers regardless of their periodontal condition (healthy, gingivitis, or periodontitis). Furthermore, dysbiosis in smokers remained significant even after the resolution of experimentally-induced gingivitis and following recovery of clinical signs of periodontitis by non-surgical periodontal treatment and over 3 months post-therapy. Smoking cessation in periodontitis patients may be beneficial in terms of compositional changes of the subgingival microbiome toward a healthy subgingival microbial community. Further studies are required to elucidate the impact of tobacco intervention on the prevention of recurrence of periodontal destruction for the full achievement of tobacco interventions in dental settings.

### Conflicts of interest

The authors have no conflicts of interest directly relevant to the content of this article.

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